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Applicant:
(Name and address)

Copenhagen Biotech Assets ApS
Karlskov Bakke 7
Ganløse
DK-3660 Stenløse
Denmark

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Bo Z. Tidemann

PATENT- OG VAREMÆRKESTYRELSEN

Composite peptide compounds for diagnosis and treatment of diseases caused by prion proteins

Field of the invention

The present invention concerns conjugates comprising two or more peptides or peptide fragments optionally linked to a backbone, and the peptides or peptide fragments are spatially positioned relative to each other so that they together mimic the tertiary structure of one or more PrP^{Sc}-specific epitopes as evidenced by the test described herein.

In other words, the conjugates comprise combinations of two or more different synthetic peptides or peptide fragments coupled to a backbone such as a backbone peptide, and the two or more peptides or peptide fragments mimic structural epitopes in the pathogenic form of the prion protein. The invention also relates to the use of such conjugates as immunogens for the production of antibodies that specifically bind to the pathogenic form of the prion protein. In a specific embodiment, the peptides or peptide fragments comprise prion-related peptides bound to a non-dendritic lipopeptide backbone. Preferred uses include use in diagnostic assays of the composite compound peptides themselves as well as antibodies produced against them and uses as vaccine immunogens for the profylactic protection and therapeutic treatment of humans against transmissible prion disease.

Background of the invention

Prion diseases are peculiar, inevitably deadly neurodegenerative diseases that can occur spontaneously, in inherited forms and by transmission (for a recent review see Prusiner, S. B. Prion Biology and Diseases, Cold Spring Harbor Laboratory Press, New York, 1999). Transmitted prion diseases include Transmissible spongiform encephalopathies (TSE) that are characterised by the occurrence of transmissible protein aggregates (prions) in the brain of the affected individual. The diseases associated with such aggregates encompass sporadic, iatrogenic and familial Creutzfeldt-Jakobs disease (CJD), kuru, Gerstmann-Straussler-Sheinker (GSS) disease, and fatal familial insomnia (FFI) in humans and bovine spongiform encephalopathy (BSE) in cattle, scrapie in sheep, chronic wasting disease (CWD) in deer and elk, and transmissible encephalopathies in mink, cat and other animals.

These deadly diseases all have very long incubation periods (up to several years) and are characterised by the absence of defensive responses (immune responses, inflammatory

responses or any other response), except a certain degree of activation of glial cells in the brain quite late in the incubation period.

It is a central dogma of these diseases that they are caused by a misfolded conformer of a normal host protein (the prion proteins, PrP) that when misfolded is also able to transmit disease, i.e. induce the abberant folding on normal prion proteins, eventually leading to disease and pathological changes in the affected individual. Although the participation of the host PrP is central to propagation of disease, prion proteins have a peculiar ability of retaining or transmitting strain characteristics (with specific incubation times, histopathology and glycosylation) even upon the "infection" of a new host. Transspecies infection occurs rarely in nature – with the very notable exception of bovine spongiform encephalopathy, which very probably can be transmitted orally to human subjects and cause new variant CJD - but disease can often be induced by intracerebral injection of pathogenic prion proteins into a new host of a new species; for example scrapie prions (prion from the wellknown prion disease in sheep) can easily be propagated in hamster brains.

Different prion disease show different prion deposition patterns in the host; for example, scrapie prions can normally be found in the brain and in the central nervous system in addition to the peripheral nerve system and in immunological organs as e.g. lymph nodes as well as in the blood and in the cerebrospinal fluid. By contrast, BSE prions only occur in the brain and in a few parts of the spinal cord; in the human counterpart of BSE, nvCJD, the pathogenic prion proteins are found also in peripheral tissues in addition to it's presence in the brain.

As no host factors have been described to be100% specific for infection with pathogenic prion proteins, diagnosis of the disease relies on the detection of the specific, disease-associated proteins found in the prions. As mentioned above, such proteins have been found to be a disease-specific conformer of a normal protein, the prion protein (PrP^C). PrP is a small, membrane-anchored glycoprotein with two N-bound glycans and a C-terminal glycosylphophatidylinositol anchor. It has been found and sequenced in a number of species and the tertiary structure has been determined by NMR- or X-ray spectroscopy for human, murine, hamster and bovine PrP^C. PrP^C has a very unusual ability to switch between a normal conformation or structure and a pathogenic, disease-associated and transmissible conformation or structure. Such abnormally folded prion proteins are denoted PrP^{Sc}. PrP^{Sc} is remarkable by being extremely stable and very resistant towards proteases, by being able to cause disease and by being able to transmit disease.

Proteinase K treatment of PrP^{Sc}, employing conditions degrading totally most other proteins, including PrP^{C}, leads to a shortened, protease resistant PrP^{Sc} protein molecule weighing 27-30 kD (PrP^{C} has a molecular weight of 33-35 kD). Apart from this and in contrast to PrP^{C}, PrP^{Sc} is insoluble if not treated with harsh chaotropic chemicals and is clearly folded differently from PrP^{C} although the details of the folding of PrP^{Sc} are not yet known.

There are no differences between the amino acid sequences of PrP^{Sc} and PrP^{C}. Thus, prion diseases are linked to the occurrence of pathogenic tertiary structures of otherwise normal host proteins. With the transmissible forms of prion diseases these proteins, in their patogenic conformer in addition have the ability to transmit disease to a normal host by exposure to the misfolded protein.

BSE emerged in the mid-eighties in Great Britain and quite rapidly evolved into a major epizootic disease, leading to clinical disease in a huge number of cattle (approximately 200,000 until now) with around 20,000 new cases pr. year in the peak years around 1992. The appearance of a new, human prion encephalopathy, nvCJD gave rise to the suspicion that this new human disease was due to exposure to the cattle pathogen and although no definitive proof of this has been obtained this is now accepted as a distinct possibility. Therefore it is highly desirable to identify and remove from human consumption animals and meat containing BSE prions. To be able to do this very sensitive, specific and preferably rapid diagnostic assays for detection of PrP^{Sc} in easily obtained samples are needed. Traditional dignosis is performed by immunohistochemistry on brain sections in combination with histopathology looking for neuropathological changes. These are quite lenghty methods (weeks) and rely on brain tissue from dead animals.

Recently, with the BSE-spurred interest in diagnostic methods for prion protein detection a number of other methods, based on the use of PrP-specific antibodies in combination with a proteinase K-degradation have evolved, three of those having been evaluated in the EU. These three EU-validated "rapid" tests (Prionics Western blotting, Enfer (Abbott) ELISA, CEA (BioRad) ELISA) all got 100% sensitivity and 100% specificity in the EU-testing (taking place in May 1999). The samples used for this validation were:
336 samples from 300 positive animals (clinical, confirmed cases from UK) and
1064 samples from 1000 negative animals (healthy, adult bovines from New Zealand).

This means that these tests can correctly identify clinically ill animals, i.e. they do determine the presence of a disease-specific component in brain (or spinal cord) samples

from clinically ill animals. Conversely, healthy animals do not present this component in brain (or spinal cord) samples and hence are not positive in these tests. It is claimed for all three tests (but this was not evaluated in the EU testing) that they can detect the presence of pathogenic prion proteins from around two months before the clinical disease appears. This, however, is far from ideal, as the real aim of a diagnostic test for a contagious disease is to be able to show the presence of the transmittable agent as soon as it is present in the animal and thus able to transmit to another animal. All three tests fail in this respect as they only show the presence of pathogenic prion protein very late in the infection process, i.e. close to the onset of clinical disease and years after the introduction of the agent into the animal. Ultimatively, the sensitivity should be also high enough for the test to be able to show the presence of infectious prions in asymptomatic carrier animals including other species of animals (pigs, chickens, etc.) which may be able to – but has not been shown to be able to - carry the infection.

Description of the invention

As mentioned above, the known tests suffer from a number of drawbacks. Thus, a major improvement would be to avoid a protease treatment step as this slows down and complicates the assay, decreases the sensitiviy of the assay and precludes detection of misfolded and pathogenic but not protease-resistant forms of PrP; such hypothetical forms may occur during the early phases of infection before enough misfolded PrP has been formed to protease-resistant PrP^{Sc}-aggregates to occur.

In one aspect, the present invention provides an improved test for determination of the presence of PrP^{Sc}. The test involves the use of a conjugate comprising two or more peptides or peptide fragments linked to a backbone in such a manner that the fragments are spatially positioned relative to each other so that they together mimic the tertiary structure of one or more PrP^{Sc}-specific epitopes as evidenced by the test described herein.

Given the inadequacies of the current tests there is a need for an improved test, and any improvement is of significant interest with regards to consumer safety. An argument in favour of more sensitive tests is that such tests will allow a much more selective culling and destruction strategy to be employed.

The test should ideally be able to detect "infection" with (or "incubation of") pathogenic prion proteins in the pre-exponential phase. Concentrations of infectious material in this

phase is not known and will depend on the source of the sample material, but it is clearly going to be very low and probably absent in some types of samples. Any improvement of sensitivity, however, is of interest, as this will allow the consideration of using other types of samples than brain samples.

The possibility of using blood or other types of "non-invasively sampled" material will be a major improvement compared to existing methods and will totally change the surveillance strategies applicable for the control of BSE. As stated above such a method will be dependend on higher assay sensitivity.

There is great concern that BSE hide as an unrecognised infection in sheep or as an infection in sheep classified as scrapie (which is not a human pathogen) and there is thus great interest in using a test that will discriminate between BSE and scrapie.

Rapidity is also a major concern, especially for normally slaughtered animals which are to be declared BSE-free. A rapid and sensitive test will pave the way for testing all slaughtered animals adding to consumer safety and increasing the value of the surveillance. Furthermore, the ideal assay should have a high capacity (high throughput), be easy to use, rapid and quantitative.

WO93/11155 describes how it is possible to produce antibodies against parts (synthetic of genetically engineered peptides) of the PrP-sequence thereby obtaining PrP-binding molecules that are useful for diagnosis, prophylaxis and treatment of PrP^{Sc} -caused disorders. While the usefulness of coupling one type or several types of such PrP-fragment to carrier molecules for immunization purposes is disclosed no mentioning is made of a composite carrier-peptide conjugate containing fragments making up PrP^{Sc}-specific epitopes. Also, the examples disclose antibodies, which do not, generally, distinguish between PrP and PrP^{Sc}. Thus the invention does not in any way teach how to produce PrP^{Sc}-specific antibodies using PrP-fragments.

WO 99/15651 describes an invention that entails the manufacturing of PrP^{Sc}-binding substances by using peptide mimotopes based on linear arrangements of one or several peptides being defined by the following substances that were found to display specific binding towards PrP^{Sc}: Monoclonal antibody 15B3, recombinant bovine PrP (rboPrP) and Congo Red. A set of four peptides defined by peptide mapping of the conformational epitopes corresponding to MAb 15B3 and rboPrP (which bind to the same epitope specific for PrP^{Sc}) is claimed as is another set of 5 peptides being mapped as Congo Red binders.

It is proposed that linear combinations of these peptides will lead to antigens that are specific to PrP^{Sc} and thus will be useful for diagnosis and therapy as well as for detection of substances binding specifically to PrP^{Sc}. However, there is no teachig as to how to achieve an arrangement of these peptide building blocks in a composite epitope, which is PrP^{Sc}-specific.

By contrast, and by utilising a non-conventional way of constructing conjugates, conformationally stabilised peptide epitopes, the present invention provides a method that by controlled spacing in a non-dendritic backbone peptide conjugate brings the peptide building blocks into close proximity to each other in space, resembling the arrangement of the peptide units found in the PrP^{Sc} -protein and allowing peptide-to-peptide stabilization of the conformation of the individual peptides.

Definitions

In the present context the term *"prion"* means a proteinaceous infectious particle, i.e. the infectious agent of prion diseases.

The term *"PrP^{C}"* denotes the prion protein, a normal host protein having a molecular weight 33-35 kDa, fully digestible by proteinase K.

The term *"PrP^{Sc}"* denotes the abnormally folded and pathogenic form of the prion protein, being trimmed to a 27-30 kDa fragment by proteinase K-treatment.

The term *"non-dendritic peptide backbone"* denotes a synthetic peptide having a defined number of derivatization points in defined positions along its peptide chains, and the derivatization points are functional groups of specific amino acid residues in the peptide chain. Specific backbones particularly suitable for use for immunization purposes are the non-dendritic peptide backbones as defined in WO 97/38011.

The term "bovine PrP" denotes a peptide identified as follows (SEQ ID No:1) (whole sequence, including signal peptide (underlined); numbering is according to this sequence throughout the text) or polymorphs thereof:

MVKSHIGSWI LVLFVAMWSD VGLCKKRPKP GGGWNTGGSR YPGQGSPGGN
RYPPQGGGGW GQPHGGGWGQ PHGGGWGQPH GGGWGQPHGG GWGQPHGGGG
WGQGGTHGQW NKPSKPKTNM KHVAGAAAAG AVVGGLGGYM LGSAMSRPLI
HFGSDYEDRY YRENMHRYPN QVYYRPVDQY SNQNNFVHDC VNITVKEHTV
TTTTKGENFT ETDIKMMERV VEQMCITQYQ RESQAYYQRG ASVILFSSPP VILLISFLIF
LIVG

Analogously, SEQ ID No: 2 relates to Ovine PrP and SEQ ID No:3 to Human PrP. The terms also include any polymorphs thereof.

Abbreviations

BMPS:	N-[β-maleimidopropyloxy]succinimide ester
bo:	bovine
BSA:	bovine serum albumin
BSE:	bovine spongiform encephalopathy
CJD:	Creutzfeldt-Jakob disease
CWD:	chronic wasting disease
ELISA:	enzyme-linked immunosorbent assay
ESI:	Electrospray ionization
FFI:	fatal familial insomnia
Fmoc:	fluorenylmethoxycarbonyl
GSS:	Gerstman-Sträussler-Scheinker disease
HOBt:	hydroxybenzotriazole
HPLC:	high pressure liquid chromatography
MS:	mass spectrometry
Mtt:	methyltrityl
NMP:	*N*-methyl pyrrolidone
Npys:	3-nitro-2-pyridinesulfenyl
nvCJD:	new variant CJD
OD:	optical density
OPD:	ortho phenylene diamine
Pbf:	2,2,4,6,7-pentamethyl dihydrobenzofuransulfonyl
PBS:	phosphate-buffered saline
PrP:	prion protein (no specific conformer implied)
SPDP:	3-(2-pyridyldithio)propionic acid hydroxysuccinimide ester
TBS:	Tris-buffered saline
TBTU:	2-(1-H-benzotriazole-1-yl)-1,1,3,3-tetramethyluronium tetrafluoroborate
TFA:	trifluoroacetic acid
TNBS:	trinitrobenzenesulfonic acid
TSE:	transmissible spongiform encephalopathy

Peptides or peptide fragments

In one aspect, the invention relates to a conjugate, wherein two or more peptides or peptided fragments optionally are linked to a backbone, and the fragments are spatially positioned relative to each other so that they together mimic the tertiary structure of one or more PrP^{Sc}-specific epitopes as evidenced by the following test. The test is made in order to secure that the conjugate has the desired structure to enable formation of antibodies specifically directed to PrP^{Sc} without recognizing PrP^{c}. The test involves the use of two samples (a positive and a control). The first sample (positive sample) is from an animal being a carrier of PrP^{Sc} and the second sample (control) is from an animal of the same species as the first animal being a carrier of PrP^{c} but a non-carrier of PrP^{Sc} (healthy non-carrier). The test comprises detection of any PrP^{Sc} in the samples by i) contacting the samples with an antibody obtained by immunizing an animal with the conjugate, ii) measuring any PrP^{Sc} that is bound to the antibody, the test being carried out without any use of Proteinase K.

More specifically, in a conjugate according to the invention the peptides or peptide fragments comprise from 2 to 150 amino acids such as from 4 to 100, from 4 to 75, from 4 to 60, from 4 to 50 or from 4 to 40 amino acids.

In a conjugate according to a specific embodiment of the invention at least one of the two or more peptides or peptide fragments is a prion peptide or a prion peptide fragment.

Furthermore, as it appears from the examples herein, in a conjugate according to the invention all peptides or peptide fragments linked to a backbone are prion peptides or prion peptide fragments. The primary structure of such prion structures or prion peptide fragments may correspond to a bovine PrP SEQ. ID No. 1, a ovine PrP SEQ. ID No. 2, a human PrP SEQ. ID No. 3, or polymorphs or fragments thereof.

Alternatively, the prion peptide or prion peptide fragment has a primary structure corresponding to a mouse PrP of a mice, a rat, a pig, a human, a sheep, a cow, a hamster, a mule deer, a white tailed deer and a Rocky Mountain elk, or polymorphs or fragments thereof.

Surprisingly, the composite peptide compounds of the present invention can be used for immunization of animals in which the PrP gene has not been removed or modified, resulting in highly reactive and specific antibodies that can react with the pathogenic

conformers of PrP, i.e. PrP^{Sc}. The composite peptide compounds of this invention are constructed in such a manner that the different peptides that are included in the compound collectively represent a conformational epitope corresponding to a conformational (non-linear) epitope only found in the abnormally folded form of PrP.

As described above the defining difference between the normal prion protein, PrP and the abnormal, pathogenic prion protein, PrP^{Sc} at the molecular level is a difference in the folding at the secondary and tertiary structure levels of the PrP protein whereas there are no differences in the amino acid sequence. This results in a protein with pronounced differences in physico-chemical features especially big increases in insolubility and protease resistance and also biological effects as neurotoxicity and – given the right circumstances – the ability to bind to other PrP protein molecules and causing them to misfold. No structure has been determined experimentally for the PrP^{Sc} form, but it is however known that the conformational changes enclose an increase in β-structure on the expense of α-structure as well as tertiary structure changes thought to occur mainly at the N-terminal part of the protease resistant part of PrP^{Sc}. However, until now only one such PrP^{Sc}-specific conformational epitope (15B3) has been identified and mapped to three different parts of the polypeptide chain that are obviously brought into close proximity of each other in PrP^{Sc} in contrast to the case in PrP^{C} where these parts of the polypeptide chain are widely separated. Also an assembled epitope binding to the amyloid-specific dye Congo Red has been mapped to five parts of the PrP polypeptide chain.

It is to be expected that many more PrP^{Sc}-specific epitopes exist, all of them, per definition conformational (non-linear), as any linear epitope will also exist in PrP^{C}.

As the accurate three-dimensional structure of PrP^{Sc} is not known at present it is not possible to produce structural analogues by conventional methods and conventional methods for producing and displaying peptides as e.g. described in WO 98/37210 and WO 93/15651 do not allow such structural epitopes to be mimicked by peptides. The shortcomings of these methods are due to the fact that only linear arrangements of two or more different peptides are described, precluding the desired structural mimicry of the conformational epitopes that are specific for PrP^{Sc} and which are constituted by a composite of a number of peptide stretches from different parts of the polypeptide chain.

It is an object of the invention to provide such composite peptide epitopes in a form where they are conformationally well-defined i.e. conformationally stabilised and at the same time soluble and preferably immunogenic by administration with adjuvants known to a person skilled in the art of animal and human vaccination. In the invention described herein this is achieved by coupling minimal peptides that constitute a PrP^{Sc} specific conformational epitope to a non-dendritic peptide backbone. The resulting derivatised

non-dendritic peptide backbone is soluble and conformationally stable and the compound is well suited for immunization.

It is envisaged that the surprising finding of the ability of a normal animal to mount an antibody response against the PrP^{Sc} mimicking composite peptide compounds of the present invention are due to the lack of presence of such PrP^{Sc}-specific conformational epitopes in such animals. Thus, the encounter of such an animal with the conformationally stabilised PrP^{Sc} mimicking composite peptide compound of the present invention leads to a host reaction directed against the "foreign", intruding structure. By contrast the conventional dogma is that it is very hard to make normal animals produce antibodies against PrP, including PrP^{Sc} as this is not seen as "foreign" by the animal, and thus it is normally considered necessary to use $PrP^{0/0}$ animals for this purpose. By contrast, the immunization of normal animals with the structure-stabilised composite peptide compounds of the present invention takes advantage of the exact fact that PrP is not "foreign" to a normal animal and therefore such an animal will produce antibodies solely against structures that are not found in normal PrP which is exactly what the composite peptide compounds represent. It is furthermore envisaged that the inability of a normal animal to produce antibodies against PrP^{Sc} particles is due to the insolubility of natural PrP^{Sc} making its removal from the body a job of other parts of the immune system not including antibodies. In contrast, the composite peptide compounds of the present invention in addition to being conformationally stable are carefully designed to be fully soluble in aqueous, physiological buffers and are therefore fully able to induce antibody production in the immunized host.

Also, the peptide compounds can be used as such, after appropriate labeling for the detection of PrP^{Sc} as it was found that a certain class of claimed compounds of this type bound specifically to this form of the molecule, changing it's own conformation upon the binding of PrP^{Sc}

The conjugates of the present invention comprises a peptide backbone whereon peptides corresponding to PrP-segments are coupled to form an epitope mimicking an epitope found in PrP^{Sc}. The peptide backbone may be a non-dendritic peptide carrier of the type disclosed in WO9738011 having two or more attachment points onto which the PrP-peptides are coupled. The peptides coupled to the backbone are preferably coupled covalently in a controlled way by methods known to a person skilled in the art of peptide chemistry and as detailed below and in the examples and may comprise one or several of

the following PrP-derived peptides, and peptides comprising the following peptides and mixtures thereof:

(numbering is for bovine PrP; it is to be understood that any of the given bovine PrP-peptides can be substituted by the equivalent peptide from PrP of another species, especially humans and ovine)

SEQ ID NO:4	bo88-104: HGG GWGQPHGGGG WGQG
SEQ ID NO:5	bo94-105: QPHGGGG WGQGG
SEQ ID NO:6	bo100-111: G WGQGGTHGQW N
SEQ ID NO:7	bo101-115: WGQGGTHGQW NKPSK
SEQ ID NO:8	bo103-121: QGGTHGQW NKPSKPKTNM K
SEQ ID NO:9	bo134-151: GGLGGYM LGSAMSRPLI H
SEQ ID NO:10	bo173-186: YYRPVDQY SNQNNF
SEQ ID NO:11	bo217-229: MERV VEQMCITQY
SEQ ID NO:12	bo229-247: YQ RESQAYYQRG ASVILFS
SEQ ID NO:13	bo231-242: RESQAYYQRG AS

Especially preferred are any peptide from the following regions of PrP as they constitute regions that are selectively exposed in PrP^{Sc} and not in PrP^{C}:

SEQ ID NO:14	bo114-123: GGTHGQW NKPSKPKTNM KHV
SEQ ID NO:15	bo153-171: GSDYEDRY YRENMHRYPN Q
SEQ ID NO:16	bo139-176: YM LGSAMSRPLI HFGSDYEDRY YRENMHRYPN QVYYRP

In another embodiment the above three peptides are synthesized on β-strand inducing building blocks known in the art and available commercially, including dibenzofuran turn mimics, before coupling to the backbone molecule. One example of such as construct is: SAMSRPLIHFG-dib-SDYEDRYYR, in which "dib" represents a 4(2-aminoethyl) 6-dibenzofuranpropionic acid residue, and the amino acids representing region 143-162 (SEQ ID NO:17) in bo PrP.

Other examples of this kind of peptides include:

SEQ ID NO:18	bo114-123: GGTHGQW NKPSKPKTNM KHV
SEQ ID NO:19	bo153-171: GSDYEDRY YRENMHRYPN Q
SEQ ID NO:20	bo139-176: YM LGSAMSRPLI HFGSDYEDRY YRENMHRYPN QVYYRP

all of which are synthesized with a β-strand inducer i the N-terminal end.

In one particular and preferred embodiment the following three 15B3 binding peptides are coupled together, one copy of each peptide on the backone in a controlled orientation ensuring their side-by-side positioning:

SEQ ID NO:21 bo153-159: GSDYEDR
SEQ ID NO:22 bo173-181: YYRPVDQYS
SEQ ID NO:23 bo226-237: ITQYQRESQAYY

Another equally preferred embodiment uses the following peptides in a similar arrangement:

SEQ ID NO:24 bo41-44: YPGQ and peptides from PrP comprising this fragment.

SEQ ID NO:25 bo octarepeat: GWGQPHGGGWGQPHGG, and peptides being shorter parts of this sequence.

Also preferred is the use of the following peptides or shorter parts thereof in a composite peptide construct:

SEQ ID NO:25A
GQGGTHGQW NKPSKPKTNM KHVAGAAAAG AVVGGLGGYM LGSAMSRPLI HF DC VNITVKEHTV TTTTKGENFT ETDIKMMERV VEQMCITQYQ RESQAYYQRG AS
(bo102-152 + 199-242)

SEQ ID NO:25B
SRPLI HF DC VNITVKEHTV TTTTKGENFT ETDIKMMERV VEQMCITQYQ RESQAY
(bo146-152 + 199-236)

SEQ ID NO:25C
SRPLI HF DC VNITVKEHTV TTTTKGENFT ETDIKMMERV VEQMCITQYQ RE
(bo146-152 + 199-232)

SEQ ID NO:25D
SRPLI HF DC VNITVKEHTV TTTTKGENFT ETDIKMMERV VEQMCITQYQ
(bo146-152 + 199-230)

SEQ ID NO:25E
SRPLI HF DC VNITVKEHTV TTTTKGENFT ETDIKMMERV VEQMCITQ

(bo146-152 + 199-228)

SEQ ID NO:25F
SRPLI HF DC VNITVKEHTV TTTTKGENFT ETDIKMMERV VEQMCIT
(bo146-152 + 199-237)

Specifically for use in a "peptide-based assay" the following peptides are used together with structure relaxing substances (as e.g. the $[RG_2]n$ peptide (n=2-4), or K_n (n=3-6)) in an optimal solvent ensuring the highest possible conformational sensitivity of the peptide to contact with PrP^{Sc} as described in detail below:

SEQ ID NO:26 Peptides derived from bo90-145: G GWGQPHGGGG WGQGGTHGQW NKPSKPKTNM KHVAGAAAAG AVVGGLGGYM LGSAM

Additionals peptides that are useful for inclusion in a composite peptide compound include:

SEQ ID NO:27	bo102-113: GQGGTHGQWNKP
SEQ ID NO:28	bo108-119: GQWNKPSKPKTN
SEQ ID NO:29	bo134-151: GGLGGYMLGSAMSRPLIH
SEQ ID NO:30	bo136-154: LGGYMLGSAMSRPLIHFGS
SEQ ID NO:31	bo231-242: RESQAYYQRGAS
SEQ ID NO:32	bo97-112: GGGGWGQGGTHGQWNK
SEQ ID NO:33	bo153-171: GSDYEDRYYRENMHRYPNQ
SEQ ID NO:34	bo151-160 CGSDYEDRY
SEQ ID NO:35	bo173-181: YYRPVDQYS
SEQ ID NO:36	bo227-237: TQYQRESQAYY
SEQ ID NO:37	bo238-254: QRGASVILFSSPPVILL
SEQ ID NO:38	bo173-186: YYRPVDQYSNQNNF
SEQ ID NO:39	bo226-237: ITQYQRESQAYY
SEQ ID NO:40	bo204-215: TKGENFTETDIK
SEQ ID NO:41	bo153-160: GSDYEDRY
SEQ ID NO:42	bo155-173: DYEDRYYRE
SEQ ID NO:43	bo173-181: YYRPVDQYS
SEQ ID NO:44	bo227-240: TQYQRESQAYYQRG

Also interesting peptides are peptides being parts or fragments of the above sequences.

In a specific embodiment, at least two or the two or more peptides or peptide fragments of

a conjugate according to the invention may have identical amino acid sequences.

Backbone

In a specific embodiment, the two or more peptide or peptide fragments are linked to a backbone. The linking makes it possible to ensure that the peptide or peptide fragments are spatially positioned relative to each other so that they together mimic the tertiary structure of one or more PrP^{Sc}-specific epitopes.

In a further embodiment at least three such as 4, 5, 6, 7, 8, 9 or 10 peptides or peptide fragments are linked to a backbone.

The linear peptides are combined into a conjugate by joining them to a common backbone such as, e.g., a backbone peptide, which functions as a scaffold carrying the peptides at predefined positions, stabilising their conformation and exposing them to the environment. To function as scaffolds for different linear prion peptides, backbone peptides are manufactured, preferably by chemical synthesis, to comprise a defined number of derivatizable functional side-chain localised groups in defined positions along the peptide chain. Also useful are backbone peptides in which a part of the peptide is already constituted by a prion peptide in a suitably protected form.

Examples of such backbone peptides are given below.

In an embodiment particularly preferred for immunization purposes the backbone peptide furthermore comprises a fatty acid. A particular preferred form of backbone peptides intended for immunization purposes is a socalled non-dendritic peptide carrier as disclosed in WO 97/38011.

The conjugates according to the invention comprise a non-dendritic peptide backbone onto which the prion-specific peptides are attached. Such backbones are preferably branched lipopeptides with molecular weights below 15.000 kD and are characterized by being soluble in benign buffers (i.e. physiological aqueous buffers), by displaying a well-defined (i.e. stable) conformation, and by being highly immunogenic. The non-dendritic backbone peptide part can contain a number of different attachment points for coupling prion peptides and they can be designed with different protecting groups with different and complementary chemical stabilities to allow the selective deprotection of a selected number of attachment point at selected positions in the non-dendritic peptide backbone. This allows the coupling of specific peptides at specific positions to be performed. Anon-

dendritic backbone peptide suitable for the present use is such backbones as disclosed in WO 97/38011. They are synthesized by solid phase peptide chemical synthesis and can be used for coupling of other substance while deprotected or selectively deprotected and still attached to the solid phase.

Examples of preferred non-dendritic backbone peptides include:

a) *palm*-KVAKLEAKVAKLEAKVAKLEAKG

b) *palm* -VACLEAKVACLEAKVACLEAKGKGKG

c) *palm* -VAKLEAKVACLEAKVACKGKG

d) *palm* -VAKLEAKVACLEAKVAKLEAKVAC

- in which K is selectively side-chain deprotectable compared to the other protected amino acid residues present in the peptide.

and

KRGGKRGGK-(*palm)*

palm-VAKLEAKVACLEAKVACKG K G

palm-VAKLEAKVACLEAKVAKLEAKVACKG KG

- in which K is selectively side-chain deprotectable compared to the other protected amino acid residues present in the peptide.

It is also an object of the present invention to provide conjugates comprising non-dendritic backbone peptides, which are not lipidated.

Additional examples of such conjugates are compounds in which the backone peptide is itself a prion peptide carrying another prion peptide. A specifically preferred example of this is:

GSDYEDRYYK-(*palm)* coupled to CYYRPVDQYSN

YMLGSAMSRPK-(*palm)* coupled to CGSDYEDRYYRE

These constructs are preferably coupled together through the cysteine thiol group to a thiol-reactive heterobifunctional coupling reagent (for example BMPS or SPDP) coupled to the N-terminal amino group of the other peptide.

A further object on the present invention is to provide composite compounds comprising prion peptides coupled to a backbone peptide, said prion peptides being characterised by havng included in their sequence an acessory residue stabilising the secondary structure

of said peptide. An example of such an accesory molecule is 4(2-aminoethyl) 6-dibenzofuranpropionic acid (a β-strand inducer).

As an example of how the composite peptide compounds of the invention can be produced using the non-dendritic backbone peptide of the above types, the nondendritic backbone peptide a) can be selectively deprotected on K, then derivatised with N-[β-maleimidopropyloxy]succinimide ester (BMPS), and subsequently reacted with a mixture of cysteine-containing synthetic prion peptides, leading to a composite peptide compound consisting of the non-dendritic backbone peptide molecule onto which a stoechiometric mixture of prionpeptides are coupled. In another example, employing structure b) this method is reversed, using synthetic BMPS-derivatised prion-peptides that are coupled to the deprotected cysteine side-chain thiol groups of the non-dendritic backbone peptide. In both cases a composite peptide compound containing an equal mixture of (bo145-151), (bo165-173) and (bo219-229) is preferred.

In c) and d) K is selectively deprotected and the ϵ-amino group used as an anchoring point for the stepwise chemical synthesis of one prion peptide, using a standard protocol with the nondendritic backbone peptide still attached to its solid phase. Hereafter the whole peptide is deprotected and used for coupling of synthetic BMPS-derivatised prion peptides. In this way the exact positioning of the individual peptides are fully controlled. A particularly preferred embodiment of such composite peptide compounds is a nondendritic backbone peptide c) onto which is coupled (bo145-151) on the selectively deprotected lysine ϵ-amino group followed by BMPS-coupled (bo165-173) and BMPS-coupled (bo219-229) on the two cysteine thiol groups (mixture). A further preferred embodiment comprises the nondendritic backbone peptide d) carrying bo(145-151) on the ϵ-amino groups of the two selectively deprotected lysine residues and BMPS-coupled bo(165-173) bound through the cysteinyl thiol groups to the nondendritic backbone peptide.

It is an object of the present invention to provide PrP^{Sc} mimics that can be used to produce PrP^{Sc}-specific antibodies in laboratory animals, said antibodies intended for use in diagnostic assays for prion diseases, i.e. for sensitive detection of PrP^{Sc}. Another intended use is as vaccine immunogen for the vaccination of subjects and animals against transmissible prion diseases. As detailed above, there is a very small risk of inducing antibodies against the normal prion protein of the host as the composite peptide compounds of the invention are designed to represent conformational epitopes of PrP^{Sc} only.

Other aspects of the invention

In one particular preferred embodiment of the invention the composite peptide conjugate assumes a PrP^{Sc} specific conformation only upon the exposure to PrP^{Sc} or PrP^{Sc} aggregates. This conformation is then detected by specific antibodies or by change in fluorescence accomplished by incorporating fluorescent molecules in the composite peptide construct.

This allows the direct use of the composite peptide conjugates of the invention for detection of PrP^{Sc}. A test can be performed to ensure that the composite peptide has the desired structure in order to be able to undergo a conformational change when contacted with PrP^{Sc}, but not when contacted with PrP^{C}. The test involves the use of two samples (a positive and a control). The first sample (positive sample) contains PrP^{Sc} and the second sample (control) contains PrP^{C}. The test comprises detection of PrP^{Sc} by means of a conformational change of the conjugate by i) incubation of the conjugate in a structure-relaxing solvent with the two samples, respectively, ii) measuring any conformational change of the conjugate by conformation-specific antibodies or by detection of changes in the fluorescence of an environmentally sensitive fluorophore coupled to the conjugate.

The feasibility of using the composite peptide conjugates of the present invention as direct probes for the presence of PrP^{Sc} as opposed to PrP^{C} is based on the fact that prion-derived peptides can induce PrP^{C} to become PrP^{Sc} *in vitro* under conditions of large surplus of peptide and provided the peptide is partly unfolded (Prusiner, S., et al., 1995, U S5750361); this, in turn, means that the unfolded peptide participates directly in this transformation, initiating it by binding to PrP^{C}. Based on this observation, it is an object of the present invention to provide PrP^{C} peptide-containing composite peptide compounds mimicking parts of PrP^{C} that, upon contact with PrP^{Sc} under appropiate solvent conditions (structure relaxing solvent conditions) undergo a conformational change that can be observed through fluorescent labels on the peptide. The basic idea is to provide conformationally facilitated PrP^{C}-peptides and allowing them to interact with PrP^{Sc} in a conformationally relaxing solvent. "Conformationally facilitated" means that the PrP^{C} peptides of the compound are arranged in order to have an enhanced tendency to form a β-sheet structure, but being in a random coil in the non-provoked compound. Thus, a peptide from the conformationally sensitive part of PrP (for bo PrP this will be the 90-145 region) is brought into contact with a sample in a structure relaxing solvent, or employing other forms of structure relaxing treatments (one preferred example being sonication as reported by Saborio et al. (Saborio, G.P., 2001, Nature 411, 810-813)). If the sample contains PrP^{Sc} this will influence the conformation of the composite peptide and this conformational change can, in turn, be detected by the conformation-specific antibodies of

the present invention or through changes in the fluorescence of built-in and environmentally sensitive fluorophor-quench pairs. The effect can be enhanced by inserting structure-relaxing peptide sequences into the prion peptides; such peptide sequences include $[RG_2]_n$, where n=2-4, as well as K_n, where n=3-6, and they are preferably placed in one end of the prion peptide and preferably in the end being attached to the backbone peptide. The final design of a peptide compound of this type will be characterised by a subtle balance between structure stabilization and relaxation to allow the conformational shift occurring upon exposure to PrP^{Sc} to become as big as possible. Also the influence of PrP^{C} in the sample will have to be taken into consideration, and conditions will have to be employed which minimize binding of the peptide to this form of the prion protein.

It is furthermore expected that an assay of this type can be rendered species specific by choosing species specific sequences from the relevant region of the prion protein. This is possible because it is to be expected that the conformational sensitivity of a given prion peptide sequence built into a composite peptide of the present invention will be higher towards PrP^{Sc} from the species in question or, if present in another species, higher towards the specific PrP^{Sc} type ("strain") formed in response to the original PrP^{Sc} in this particular species. This principle of "strain persistency" has been observed and reported in the scientific literature in a number of cases (for example various scrapie PrP^{Sc} in hamsters, see e.g. Safar, J., et al., 2000, Arch. Virol. Suppl. 16, 227-235) and the principle and its implications for the composite peptide assay is outlined in Figure 4.

It is also the object of the present invention to provide antigens that mimic parts of the abnormally folded prion protein, PrP^{Sc}, that are not found in the normal form of the prion protein, PrP^{C}. The invention also encompasses the use of such antigens for the production of antibodies that are specific for PrP^{Sc}, by immunization with said antigens and diagnostic assays employing such antigens and antibodies.

The invention also describes a metod for the manufacture of such antigens in which a conjugate having a polypeptide chain is produced. The polypeptide chain corresponds to an assembled epitope at the surface of the PrP^{Sc} and the epitope is not being present in PrP^{C}. The conjugate is generally produced by chemical peptide synthesis by chemically combining a number of linear peptides, each corresponding to a linear stretch of amino acids in the prion protein into a composite molecule. The linear peptide building blocks can be produced by chemical peptide synthesis or optionally by expression by a recombinant expression system.

The conjugates of the invention can be tested to secure that the conjugates has the desired structure to enable formation of antibodies specifically directed to PrP^{Sc} without recognizing PrP^{C}. The test involves the use of two samples (a positive and a control). The first sample (positive sample) is from an animal being a carrier of PrP^{Sc} and the second sample (control) is from an animal of the same species as the first animal being a carrier of PrP^{C} but a non-carrier of PrP^{Sc} (healthy non-carrier animal). The test comprises detection of any PrP^{Sc} in the samples by i) contacting the samples with an antibody obtained by immunizing an animal with the conjugate, ii) measuring any PrP^{Sc} that is bound to the antibody, the test being carried out without any use of Proteinase K.

Also, by providing a sensitive means of detection of malfolded prion proteins without using protease resistance as a parameter, the present invention allows the detection of PrP^{Sc} in biological material where it is present in trace amounts only, e.g. in cerebrospinal fluid, blood, urine, saliva, tear fluid etc.

An important feature of the invention is the possibility of achieving a satisfactory degree of species specificity by using the exact amino acid sequence of the species in question. It is also possible, however, to construct conjugates that are not specific for one species but will correspond to prion proteins from a number of species by constructing the peptides using consensus or homologous amino acid stretches of the prion protein, or by providing a mixture of molecules each representing a different form of the prion protein.

Furthermore, the conjugates provided herein can be used for the screening of various substances, including combinatorial chemical libraries, for PrP^{Sc} –binders. This can be achieved by assays employing blocking of PrP^{Sc}-specific antibodies or blocking of PrP^{Sc}-sensitive composite peptide compounds. Substances defined by such methods are useful leads for the generation of drugs that block the change of PrP^{C} into of PrP^{Sc}.

The cojugate of the present invention are PrP^{Sc}-mimics that can be easily produced chemically in large scale, and are non-infectious and easy to handle by anyone skilled in the art of peptide chemistry and immunology. Preferred uses include directly as reporter substances in PrP^{Sc}-assays, utilizing either antibody detection or a flurophor signal generation detection system. Also preferred is the use of such compounds for the production of diagnostic antibodies in laboratory rodents, and the use of said antibodies to specifically detect PrP^{Sc} in a high background of PrP^{C}. A highly preferred use of assays based on such antibodies is for the detection of minute amounts of PrP^{Sc} in ante-mortem

types of samples including blood, saliva, urine and the like. Another preferred use of such compounds is for production of vaccines as well as therapy against transmissible prion diseases targeting specifically the PrP^{Sc} conformer of the prion protein. Apart from these medical uses it is clear that the PrP^{Sc}-specific antibodies and composite peptide compounds of the present invention will also be highly useful for research purposes, one particular preferred use being the high-throughput screening of pharmaceuticals interfering with PrP^{Sc} formation or directly destroying PrP^{Sc} complexes.

Still further aspects of the invention appear from the appended claims.

Legends to figures

Figure 1

The N-terminal part of the globular domain of PrP^{C} is schematically depicted to the left, indicating the presence of helix 1 and the two-beta strand sheet as experimentally determined to constitute the structure of PrP^{C} in this region. After transformation into PrP^{Sc} the resulting putative structure shown to the right of the arrow occurs, transforming the original helix 1 into two additional beta strands teaming up with the original two beta-strands (1 and 4) to form a four beta-strand sheet as indicated. Two parts of the 15B3 epitope (specific for PrP^{Sc}) are marked by the boxes.

To the right in the figure is indicated a PrP^{Sc}-specific peptide mimic, employing a beta-strand promoting turn-residue (DIB, see text). This peptide specifically mimics PrP^{Sc}, as this structure is obviously not found in PrP^{C}.

Figure 2

Left-hand side of the figure is as in Figure 1. In the middle part is indicated the positions of two peptide mimics and the positions of two 15B3-epitopes. The two peptide mimics are produced by coupling two different peptides from different parts of the chain together in opposite directions, thereby specifically mimicking PrP^{Sc}-specific structures (peptide chain directions are indicated by the small arrows, fat zig-zag line symbolizes palmitate).

Figure 3

Left-hand side of the figure is as in Figure 1. The middle part indicates the position of all PrP^{Sc}-specific 15B3-epitopes and also shows the position of helix 3 and its C-terminal extension in the PrP^{Sc} protein. To the right are indicated (from the top) positions and directions of prion peptides in a a composite peptide carrying three different prion

peptides, and two examples of composite peptides carrying two different prion peptides. In these structure the peptide representing 15B3/1 is always present as this is the PrP^{Sc}-defining part of the epitope, resulting in PrP^{Sc}-specific composite peptides when combined with any other parts of the 15B3-epitope or both parts at the same time.

Figure 4

Schematic figure depicting the relationship of prion proteins from one species (all vertically hatched objects) to prion proteins from another species (all chequered objects), the circular figure depicting PrP^{C}, i.e. the normal conformer and pathogenic conformers (PrP^{Sc}) being depicted by various quadrangles.

It is hypothesized that PrP^{C} exists in a very skewed equilibrium with various unstable PrP^{Sc} conformers (the PrP^{Sc} conformer population), one specific PrP^{Sc} conformer, however, being the preferred one in each species (the PrP^{Sc} strain characteristic for that species).

Furthermore, the figure shows how PrP^{Sc} from one species can "infect" another species leading to stabilization of unstable PrP^{Sc} into pathogenic, stable PrP^{Sc} aggregates containing PrP^{Sc} conformers of the inoculum type ("strain"). Thus, a specific PrP^{Sc} conformer preferentially binds to and stabilises the corresponding specific PrP^{Sc} conformer of the other species even if this conformer is only a minor part of the PrP^{Sc} conformer population of that species. Note that the development of PrP^{Sc} conformers of this type is slower than the development of PrP^{Sc} conformers of the type typical for the species being infected.

Also shown is how a labeled, conformationally sensitive peptide ("reporter peptide") can be used to detect PrP^{Sc} in a sample by specific binding to that PrP^{Sc} leading to a measurable change in the conformationally sensitivite label of the peptide.

As the preference for forming a particular PrP^{Sc} conformer is thought to be governed by the exact amino acid sequence of the prion protein of the species in question, a peptide built from that particular sequence will also show preference towards being bound by that particular conformer even if they are built from another amino acid sequence, i.e. from the prion protein of another species.

Thus the reporter peptide will always indicate the "strain-type" of the infecting PrP^{Sc} to the extend that this type is replicated in the host species PrP^{Sc}.

Methods

Test - immunization

As it appears from the appended claims, a conjugate according to the invention comprises two or more peptides or peptide fragments optionally linked to a backbone and the peptides or peptide fragments are spatially positioned relative to each other so that they together mimic the tertiary structure of one or more PrP^{Sc}-specific epitopes. A suitable test for demonstrating that the structure is suitable is as follows:

The test is made in order to secure that the conjugate has the desired structure to enable formation of antibodies specifically directed to PrP^{Sc} without substantially recognizing PrP^{C}. The test involves the use of two samples (a positive and a control). The first sample (positive sample) is from an animal being a carrier of PrP^{Sc} and the second sample (control) is from an animal of the same species as the first animal being a carrier of PrP^{C} but a non-carrier of PrP^{Sc}.

The test comprises detection of any PrP^{Sc} in the samples by i) contacting the samples with an antibody obtained by immunizing an animal with the conjugate, ii) measuring any PrP^{Sc} that is bound to the antibody, the test being carried out without any use of Proteinase K.

Test - peptide assay

In another embodiment the invention relates to a conjugate comprising two or more peptides or peptide fragments optionally linked to a backbone and the peptides or peptide fragments are spatially positioned relative to each other so that they together mimic the tertiary structure of and have the same or a higher degree of conformational sensitivity to PrP^{Sc} as one or more conformationally sensitive regions of PrP, A suitable test to demonstrate the correct structure is given in the following:

The test is made to secure that the conjugate has the desired structure in order to be able to perform a conformational change when contacted with PrP^{Sc}, but not or at least not to the same extent when contacted with PrP^{C}. The test involves the use of two samples (a positive and a control). The first sample (positive sample) contains PrP^{Sc} and the second sample (control) contains PrP^{C}.

The test comprises detection of any conformational change of the conjugate by i) incubation of the conjugate in a structure-relaxing solvent with the two samples, respectively, ii) measuring any conformational change of the conjugate by conformation-specific antibodies or by detection of changes in the fluorescence of an environmentally

sensitive fluorophore coupled to the conjugate.

Examples

The following examples demonstrate the feasibility of the invention but are not meant to limit the invention to the uses and the embodiments presented in the examples.

Example 1

Peptide constructs for immunization I: A conjugate according to the invention containing one PrP^{Sc}-peptide

As an example of this kind of structure, a conjugate comprising the bovine prion peptide WGQGGTHGQWNKPSK (bo101-115) coupled to the following backbone lipopeptide KRGGKRGGK-*(palm)* was synthesized and used. The BSE-peptide is coupled to the side chains of the two lysine residues that have free epsilon-amino groups, forming a conjugate containing two copies of one BSE-peptide. This peptide derives from the conformationally labile N-terminal part of the globular part of PrP and is envisaged to be stabilised in a PrP^{Sc}-like conformation by its side-by-side positioning on the backbone peptide. The region in PrP comprising this peptide is furthermore the region being processed differently in different strains and species of PrP and is therefore not present in certain types of PrP^{Sc} while present in others. The example is not limited to this bovine peptide but may also be applied to the corresponding human (hu89-103: WGQGGTHSQWNKPSK) and ovine (ov93-107: WGQGGSHSQWNKPSK) peptides and may furthermore be extended to the use of the following PrP peptides being from a conformationally labile region close to that of the above peptides and with a proven species specificity derived from the subtle sequence differences between species:
bo114-123: SKPKTNMKHV
hu103-112: SKPKTNMKHM
ov106-115: SKPKTNMKHV
and peptides being part of these sequences or polymorphs thereof.

Also very useful for inclusion into composite peptides of this type are various structure-supported PrP^{Sc}-related peptides, including
bo143-162: SAMSRPLIHFG-dib-SDYEDRYYR (see Figure 1)
bo114-123: dib-GGTHGQW NKPSKPKTNM KHV
bo153-171: dib-GSDYEDRY YRENMHRYPN Q
bo139-176: YM LGSAMSRPLI HFG-dib-SDYEDRY YRENMHRYPN QVYYRP

in which *"dib"* represents a 4(2-aminoethyl) 6-dibenzofuranpropionic acid residue (Neosystem Groupe SNPE) or another beta-strand supporting residue.These peptides are characterised by having a preferential conformation, aided by the structure-support element and subsequently further supported by their side-by-side coupling to the backbone peptide, said conformation being specific for the conformation of the peptide sequence in PrP^{Sc} and not found in PrP^{C}.

Synthesis of peptides

The following is provided as an example of a particularly preferred way of synthesizing conjugates of this type.

The backbone peptide (KR(Pbf)GGKR(Pbf)GGK-(*palm*)) was synthesized by the Fmoc/tertButyl solid phase peptide synthesis strategy on a Rink-MBHA-resin from Novabiochem, using Mtt-side chain protected lysine and Pbf-side chain protected arginine in addition to glycine, all from Novabiochem. The synthesis was performed on a semiautomatic peptide synthesizer from Abimed GmbH using TBTU/HOBt *in situ* activation and 20% piperidin in NMP for Fmoc-deprotection using standard conditions (Chan, W.C., and White, P.D., 2000, Fmoc Solid Phase Peptide Synthesis, Oxford University Press). After coupling of the first K(Mtt), the Mtt group was removed by 1% TFA, 5% triisopropylsilane in NMP and the side chain amino group was then palmitoylated by acylation with palmitic acid (Merck) in the presence of TBTU and HOBt until the colour of a sample of the resin went from orange/red to white by reaction with TNBS (trinitrobenzenesulfonic acid (Sigma). Then the synthesis was resumed, initiating with a piperidine incubation (20% piperidine in NMP) to remove the Fmoc-group. After completion of the peptide, the α-amino group was acetylated by acetic anhydride followed by washing and then by removal of the two remaining Mtt-groups by TFA/triisopropylsilane as above. This deprotects the two ϵ-amino groups of the lysine residues but leaves the arginine side-chain protection intact. Then the free amino groups were reacted with BMPS (Pierce) in NMP until a negative TNBS-test was obtained, followed by cleavage of the whole peptide as the amide from the resin by 95% TFA/water with appropiate scavengers and work-up following standard procedures (Chan, W.C., and White, P.D., 2000, Fmoc Solid Phase Peptide Synthesis, Oxford University Press).

HPLC-MS (Shimadzu LCMS 2010, ESI-MS) was performed on a sample of the peptide and the molecular weight was confirmed.

For the subsequent coupling of the BSE-peptide, this was first synthesized with a N-terminal cysteine by standard Fmoc/tBu chemistry on solid phase, cleaved as the amide and worked up following standard procedures. The actual coupling was performed by dissolving the backbone peptide to 2 mg/ml in water and mixing 1 ml of this solution with 5 mg of the freeze-dried BSE-peptide and incubating for 2 hours at room temperature with stirring. This was then freeze-dried directly. In the resulting composite peptide, the BSE-peptide was bound to the backbonepeptide through a non-reducible thioether bond. In another equally appropiate procedure, Cys(Npys) was used instead of BMPS to derivatize the free ϵ-amino groups of the lysines, and the resulting backbone peptide was reacted with the BSE-peptide as above. This gave rise to a composite peptide in which the BSE-peptide was bound to the backbone peptide through a reducible disulfide bond. HPLC-MS (Shimadzu LCMS 2010, ESI-MS) was performed on a sample of both peptide compounds and the molecular weight was confirmed.

Immunization procedures

Mice, such as female 6- to 8-weeks old BALB/c mice or (CF1xBALB/c)F1 mice, were immunized 3 times with 14 days intervals with different BSE peptide constructs mixed 1+1 with Freunds adjuvant. The mice were immunized subcutaneously with 16 µg of the peptide. The mice were bled before the first immunization and 12 days after each immunization. Sera were collected from the bleedings and tested in ELISA for antibody reactivity against BSE peptides. Likewise rabbits were immunized with app. 50 µg of BSE peptide constructs.

Antibody reactivities in ELISA assays

Peptides (0.25 µg/ml) were coated to Maxisorp microtiter plates (Nunc, Roskilde, Denmark) in 100 mM $NaHCO_3$ at pH 9.6. All coatings were performed overnight at 4 °C. To avoid background reactivity with the backbone part of the composite peptides used for immunizations, coating was performed with either the free peptides or peptides coupled to a conventional carrier protein, typically ovalbumin, by maleimide-cysteine chemistry. The wells were washed four times in washing buffer (0.5 M NaCl, 3 mM KCl, 1 mM KH_2PO_4, 8 mM Na_2HPO_4, and 1% Triton X-100). This washing procedure was done after each of the following incubation steps: 1) serum samples, 1% (v/v) in incubation buffer (washingbuffer plus 15 mM bovine albumin, pH 7.2), were incubated for 1 hr at room temperature; 2) 100 µl per well of horseradish peroxidase-conjugated rabbit anti-murine IgG antibodies (DAKO, Copenhagen, Denmark) diluted in incubation buffer, were added at room temperature for 1 hr. Enzyme activities were quantitated after the addition of 100

µl per well of 1,2-phenyldiamine hydrochloride (0.67 mg/ml) (DAKO) dissolved in 100 mM citric acid-phosphate buffer, pH 5.0, containing 0.015% (v/v) H_2O_2. The reactions stopped after 30 min by adding 50 µl per well of 2.5 M H_2SO_4, and the optical densities were measured in an enzyme-linked immunosorbent assay (ELISA) scanner at 492 nm. All tests were performed in duplicate.

Serial dilution series were carried out with positive sera in order to determine the titre of positive sera. Some seropositive mice were selected for hybridoma production by standard procedures.

Validation of positive antibodies

Seropositive sera and/or monoclonal antibodies were tested by standard procedures against PrP^{Sc}-infected brain material by ELISA, filter-based assays, Western blotting or fluorescence assays, using brain material from confirmed BSE-positive cows, from scrapie-positive sheep and from CJD-diagnosed humans, depending on the peptide in question.

Positive sera or monoclonal antibodies were selected for development of a blood based PrP^{Sc}- test. This may or may not comprise an initial extraction step to extract PrP^{Sc} in sufficient quantities to allow its quantitation.

Blood based BSE test (sandwich like type)

Seropositive sera or monoclonal antibodies (10 µg/ml) were coated to Maxisorp microtiter plates (Nunc, Roskilde, Denmark) in 100 mM $NaHCO_3$ at pH 9.6. All coatings were performed overnight at 4° C. The wells were washed four times in washing buffer (0.5 M NaCl, 3 mM KCl, 1 mM KH_2PO_4, 8 mM Na_2HPO_4, and 1% Triton X-100). This washing procedure was done after each of the following incubation steps: 1) test serum samples, 1% (v/v) in incubation buffer (washing buffer plus 15 mM bovine albumin, pH 7.2), were incubated for 1 hr at room temperature; 2) 100 µl per well of biotinylated seropositive sera or monoclonal antibodies diluted in incubation buffer, were added at room temperature for 1 hr. 3) 100 µl per well of horseradish peroxidase-conjugated streptavidin (DAKO, Copenhagen, Denmark) diluted in incubation buffer, were added at room temperature for 1 hr. Enzyme activities were quantitated after the addition of 100 µl per well of 1,2-phenyldiamine hydrochloride (0.67 mg/ml) (DAKO) dissolved in 100 mM citric acid-phosphate buffer, pH 5.0, containing 0.015% (v/v) H_2O_2. The reactions stopped after 30

min by adding 50 µl per well of 2.5 M H_2SO_4, and the optical densities were measured in an enzyme-linked immunosorbent assay (ELISA) scanner at 492 nm. All tests were performed in triplicate. Various procedures were applied to amplify the signal including standard biotin-streptavidin amplification systems. Dilutions of infected material (standard) were tested on the same plate.

Blood based BSE test (competition like type)

Synthetic peptide constructs (0.25 µg/ml) were coated to Maxisorp microtiter plates (Nunc, Roskilde, Denmark) in 100 mM $NaHCO_3$ at pH 9.6. All coatings were performed overnight at 4° C. The wells were washed four times in washing buffer (0.5 M NaCl, 3 mM KCl, 1 mM KH_2PO_4, 8 mM Na_2HPO_4, and 1% Triton X-100). This washing procedure was done after each of the following incubation steps: 1) test whole blood, serum or plasma samples, 1-50% (v/v) in incubation buffer (washingbuffer plus 15 mM bovine albumin, pH 7.2) mixed with diluted biotinylated seropositive sera or monoclonal antibody, were incubated for 2 hr at 37 °C; 2) 100 µl per well of horseradish peroxidase-conjugated streptavidin (DAKO, Copenhagen, Denmark) diluted in incubation buffer, were added at room temperature for 1 hr. Enzyme activities were quantitated after the addition of 100 µl per well of 1,2-phenyldiamine hydrochloride (0.67 mg/ml) (DAKO) dissolved in 100 mM citric acid-phosphate buffer, pH 5.0, containing 0.015% (v/v) H_2O_2. The reactions stopped after 30 min by adding 50 µl per well of 2.5 M H_2SO_4, and the optical densities were measured in an enzyme-linked immunosorbent assay (ELISA) scanner at 492 nm. All tests were performed in triplicate. Seropositive sera or monoclonal antibody mixed with buffer served as a control.

Percent inhibition of seropositive sera or monoclonal antibody binding was calculated from the formula: 100-((ELISA OD_{sample}/ELISA $OD_{control}$) x 100). Significant inhibition reflects a BSE positive sample. Standard curves were obtained with infected material in serial dilutions.

Blood based BSE test (fluorescence-based)

An adequate purified polyclonal antibody or an appropiate monoclonal antibody, prepared against PrP-relevant peptides as detailed above is used in a homogenous assay where the blood sample is brought into contact with the antibody together with a fluorescently labeled derivative of the peptide antigen in question. After a short incubation period the polarization ability of the peptide-coupled florophor is then measured in fluorescence

polarization reader. As this parameter depends on the rotational freedom of the flourescent molecule, the signal from the flourescent peptide will vary depending if the peptide is bound by the antibody or if it is competed off into free solution by a specifically binding PrP^{Sc} present in the blood sample. Thus, with an adequate PrPSc-standard, the fluorescence polarisation value expresses how much PrP^{Sc} is present in the sample.

Example 2

Peptide constructs for immunization II: Conjugate containing two different PrP^{Sc}-peptides using one of the peptides as the backbone

As an example, three peptides representing different parts of the conformational 15B3 PrP^{Sc}-specific epitope reported in the litterature (Korth et al., 1997, Nature 390, 74-77) are combined to provide composite peptides representing PrP^{Sc}-specific spatial structure. These peptides derive from the region making up the four-strand β-sheet postulated to be specific for PrP^{Sc} and constituting the part of the 15B3-epitope being different in PrP^{C} and PrP^{Sc}. It should be noted that care has been taken to ensure the right orientation of the two peptides making up the coposite peptide in order to resemble exactly the arrangement of the corresponding peptides in the PrP^{Sc}-structure (see Figure 2).

It is to be understood that while the example deals with boPrP-derived peptides and hence with detection of BSE-derived PrP^{Sc}, the example is likewise applicable to human and ovine PrP^{Sc}-detection, using the equivalent sequences from these two proteins. One composite peptide comprises bmps-GSDYEDRYYK-(*palm)* (bo153-161 with an additional C-terminal lysine to which palmitate is coupled and a BMPS-labeled N-terminal) as the backbone peptide onto which CYYRPVDQYSN (bo173-182 with an additional N-terminal cysteine) is coupled. As can be seen from Figure 2 this mimics the arrangement of the peptide turn C-terminally to the second β-strand of the PrP^{Sc}-specific β-sheet and the peptide stretch C-terminally to the fourth β-strand of the PrP^{Sc}-sheet, with opposite directions of the peptide chains. Furthermore, by placing palmitate on the C-terminal amino acid of the second β-strand related peptide, the immunogen will orient itself with the β-strand four-related peptide as the outermost part of the immunogen. For this particular construct a similar composite peptide with palmitate introduced at the N-terminus of one of the two peptides or at the C-terminus of the β-strand four-related peptide will constitute other equally preferred composite peptides.

Another composite peptide contains bmpsYMLGSAMSRPK-(*palm)* (bo139-148 with an additional C-terminal lysine to which is coupled palmitate and a BMPS-labele N-terminal)

as the backbone peptide onto which CGSDYEDRYYRE (bo153-163 with an additional N-terminal cysteins) is coupled. As can be seen from Figure 2 this mimics the spatial arrangement of the first β-strand and part of β-strand 2, represented with the first peptide and the peptide turn C-terminally to the second β-strand of the PrP^{Sc}-specific β-sheet (the second peptide of the construct), again with opposite directions of the peptide chains. Here, the palmitate ensures the inwards orientation of the first peptide in the immunogen, resembling the situation in the four-strand β-sheet of the PrP^{Sc}-structure. This is the preferred position of the palmitate in this construct.

Synthesis of peptides

This was done by the same methods as described above, using Mtt-protected C-terminal lysine for the introduction of palmitate (deprotecting and acylating the ε-aminogroup before continuing synthesis). Molecular weights were confirmed by HPLC-MS (Shimadzu LC-MS 2010).

Immunizations, validations and immunoassays are then performed as described above and useful antibodies/immunoassays are expected to be obtained.

Example 3
Peptide constructs for immunization III: Conjugates containing two or more different BSE-peptides

As an example illustrating how the peptides making up the PrP^{Sc}-specific 15B3-epitope can be combined into a composite peptide as described herein the following compounds were produced:
(the three peptide sequences making up the composite 15B3 epitope (Korth et al., 1997, Nature 390, 74-77) being 15B3/1: bo153-159, GSDYEDR, 15B3/2: bo173-181, YYRPVDQYS, 15B3/3: bo226-237, ITQYQRESQAYY, respectively).

As in the other examples, the equivalent peptides from human and ovine PrP can be used to develop immunogens specific for these species.

palm-VAKLEAKVACLEAKVACKG ***K*** G

in which 15B3/1 is coupled to ***K*** and 15B3/2 and 15B3/3 are coupled to either of the two cysteines. As can be seen in Figure 3 this leads to structures in which the three 15B3-

peptides are arranged spatially in the same manner as in the PrP^{Sc}-specific 15B3-epitope, also retaining the different orientations of the peptide stretches in this epitope.

Furthermore, the backbone peptide of this example is stabilised conformationally being an amphipathic α-helix, lending further structural stabilization to the attached PrP-peptides.

palm-VAKLEAKVACLEAKVA*K*LEAKVACKG *K*G

in which 15B3/1 is coupled to ***K*** and 15B3/2 or 15B3/3 is coupled to both cysteines. As can be seen in Figure 3 the resulting structures combine 15B3/1 and 15B/2 or, in a separate composite peptide 15B3/1 and 15B3/3 in a manner resembling their arrangement and orientations in the PrP^{Sc}-specific 15B3-epitope, each composite peptide representing part of the structure (a part that in each case contains the PrP^{Sc}-specific features) and each composite peptide presenting two copies of the PrP^{Sc}-specific structure. Furthermore, the backbone peptide of this example is stabilised conformationally being an amphipathic α-helix, lending further structural stabilization to the attached PrP-peptides.

The synthesis is performed essentially as described above, starting with the backbone peptide, which is synthesized with the N-terminal palmitate before Mtt is removed when the peptide is still attached to the solid phase resin; hereafter the 15B3/1-peptide is synthesized by solid-phase synthesis on the liberated ϵ-amino groups. Then the whole peptide (now branched) is cleaved from the solid phase, in the process deprotecting the thiol groups of the cysteines (should be trityl protected). Hereonto are then coupled the N-terminally BMPS-derivatised 15B3/2 and 15B3/3 peptides as described above.

With the first peptide, a mixture of composite peptides will result, arising from different combinations of the two 15B3-peptides (2 and 3) with the already attached 15B3/1-peptide (1): 2,3,1; 3,2,1; 2,2,1; 3,3,1. All of these peptides, as well as the mixture itself are thought to be useful immunogens for the production of PrP^{Sc}-specific antibodies as outlined above.

As another example illustrating an important composite peptide, the ***K***-positions are used for the coupling of a peptide selected from those described above in the paragraph "Conjugates containing one BSE-peptide" in combination with 15B3-peptides. Specifically SAMSRPLIHFG-dib-SDYEDRYYR is synthesized on the ***K*** of the backbone peptides above and combined with any or both of 15B3/1 and 15B3/2.

Immunizations, validations and immunoassays were then performed as described above and useful antibodies/immunoassays are expected to be obtained.

Example 4

A composite peptide construct for the direct detection of PrP^{Sc}

A composite peptide compound as the one described in Example 1 above will be synthesized. As the prion peptides of this compound contain two tryptophan residues each they will exhibit an intrinsic fluoresence that will report on the relative positions of the tryptophan residues in the individual peptide chains as well as between chains.

For other prion peptides, tryptophan or tryptophan-quench pairs (one quencher building block being abbreviated aedans (= 5-((2-(tBoc)-glutamylaminoethyl)amino)naphthalene-1-sulfonic acid) from Molecular Probes) will be inserted into the prion peptides at strategic points that will reflect the conformation of the peptides.

An assay based on such compunds will be developed, using PrP^{Sc} spiked and un-spiked samples as positive and negative samples, respectively, and varying the following parameters to obtain the most sensitive peptide compound:

-type of prion peptide
-type and position of fluorescence-quench pairs
-insertion of conformation-supporting or relaxing residues
-solvent conditions for sample and/or the peptide compound
-treatment of both components and the mixture by sonication, ultrasound, heat or other means.

The assay will be analysed by fluorescence spectroscopy correcting for the background being caused by the presence of PrP^{C} in the samples.

Claims

1. A conjugate comprising two or more peptides or peptide fragments optionally linked to a backbone and the peptides or peptide fragments are spatially positioned relatively to each other so that they together mimic the tertiary structure of one or more PrP^{Sc}-specific epitopes as evidenced by the test described herein.

2. A conjugate comprising two or more peptides or peptide fragments optionally linked to a backbone and the peptides or peptide fragments are spatially positioned relatively to each other so that they together mimic the tertiary structure of and have the same or a higher degree of conformational sensitivity to PrP^{Sc} as one or more conformationally sensitive regions of PrP as evidenced by the test described herein.

3. A conjugate according to claim 1 or 2, wherein the peptides or peptide fragments comprise from 2 to 150 amino acids.

4. A conjugate according to claim 3, wherein the peptides or peptide fragments comprise from 4 to 100 such as from 4 to 75, from 4 to 60, from 4 to 50 or from 4 to 40 amino acids.

5. A conjugate according to any of the preceding claims, wherein at least one of the two or more peptides or peptide fragments is a prion peptide or a prion peptide fragment.

6. A conjugate according to claim 5, wherein all peptides or peptide fragments linked to a backbone are prion peptides or prion peptide fragments.

7. A conjugate according to claim 5 or 6, wherein the prion peptide or prion peptide fragment has a primary structure corresponding to a bovine PrP SEQ. ID No. 1, a ovine PrP SEQ. ID No. 2, a human PrP SEQ. ID No. 3, or polymorphs or fragments thereof.

8. A conjugate according to claim 5 or 6, wherein the prion peptide or prion peptide fragment has a primary structure corresponding to a mouse PrP of a mice, a rat, a pig, a human, a sheep, a cow, a hamster, a mule deer, a white tailed deer or a Rocky Mountain elk or polymorphs or fragments thereof.

9. A conjugate according to any of the preceding claims, wherein the peptides or peptide fragments are selected from the group consisting of SEQ. ID No. 4, SEQ. ID No. 5, SEQ. ID No. 6, SEQ. ID No. 7, SEQ. ID No. 8, SEQ. ID No. 9, SEQ. ID No. 10, SEQ. ID No. 11, SEQ. ID No. 12 and SEQ. ID No. 13.

10. A conjugate according to any of the preceding claims, wherein the peptides or peptide fragments are selected from the group consisting of SEQ. ID No. 14, SEQ. ID No. 15 and SEQ. ID No. 16.

11. A conjugate according to any of the preceding claims, wherein the peptides or peptide fragments are selected from the group consisting of SEQ. ID No. 17, SEQ. ID No.18, SEQ. ID No.19 and SEQ. ID No. 20.

12. A conjugate according to any of the preceding claims, wherein a β-strand inducing building block is introduced in the amino acid sequence of the peptides or peptide fragments.

13. A conjugate according to any of the preceding claims, wherein the prion peptides or peptide fragments are selected from the group consisting of SEQ. ID No. 21, SEQ. ID No. 22, SEQ. ID No. 23, SEQ. ID No. 24, SEQ. ID No. 25 and SEQ. ID No. 25 A-F.

14. A conjugate according to any of the preceding claims, wherein the prion peptides or peptide fragments have a sequence corresponding to SEQ. ID No. 26.

15. A conjugate according to any of the preceding claims, wherein the prion peptides or peptide fragments are selected from the group consisting of SEQ. ID No. 27, SEQ. ID No. 28, SEQ. ID No. 29, SEQ. ID No. 30, SEQ. ID No. 31, SEQ. ID No. 32, SEQ. ID No. 33, SEQ. ID No. 34, SEQ. ID No. 35, SEQ. ID No. 36, SEQ. ID No. 37, SEQ. ID No. 38, SEQ. ID No. 39, SEQ. ID No. 40, SEQ. ID No. 41, SEQ. ID No. 42, SEQ. ID No. 43 and SEQ. ID No. 44.

16. A conjugate according to any of the preceding claims, wherein at least two or the two or more peptides or peptide fragments have identical amino acid sequences.

17. A conjugate according to claim 1 or 2, wherein the conjugate is GSDYEDRYYK-*(palm)* or YMLGSAMSRPK-*(palm)*.

18. A conjugate according to any of the preceding claims, wherein at least three such as 4, 5, 6, 7, 8, 9 or 10 peptides or peptide fragments are linked to a backbone.

19. A conjugate according to any of the preceding claims, wherein the epitopes can bind to 15B3, Congo Red, PrP and/or PrP peptides as defined in US 5,750,362.

20. A conjugate according to any of the preceding claims, wherein a backbone is

contained in the conjugate.

21. A conjugate according to any of the preceding claims, wherein the backbone is a non-dendritic peptide backbone.

22. A conjugate according to any of the preceding claims, wherein the backbone is a lipopeptide.

23. A conjugate according to any of the preceding claims, wherein the backbone has a molecular weights of at the most about 20,000 kD such as at the most about 15,000 kD or at the most about 10,000 kD.

24. A conjugate according to any of the preceding claims, wherein the backbone is soluble in an aqueous medium.

25. A conjugate according to any of the preceding claims, wherein the backbone in itself is immune stimulating.

26. A conjugate according to any of the preceding claims, wherein the backbone has a stable conformation under physiological conditions.

27. A conjugate according to any of the preceding claims, wherein the backbone is branched or non-branched.

28. A conjugate according to any of the preceding claims, wherein the backbone contains two or more attachment points for linking of two or more peptides or peptide fragments.

29. A conjugate according to any of the preceding claims, wherein the attachment points are protected with different protecting groups having different chemical stabilities to allow selective deprotection at the attachment points.

30. A conjugate according to any of the preceding claims, wherein the backbone contains one or more Lys and/or Cys residue for linking of peptides or peptide fragments to the backbone.

31. A conjugate according to any of the preceding claims, wherein the backbone has a structure as disclosed in WO 97/38011 (Inventors: Heegaard & Høy Jakobsen).

32. A conjugate according to any of the preceding claims, wherein the backbone has a

structure selected from the group consisting of:

a) *palm* -KVAKLEAKVAKLEAKVAKLEAKG
b) *palm* -VACLEAKVACLEAKVACLEAKGKGKG
c) *palm* -VAKLEAKVACLEAKVACKGKG
d) *palm* -VAKLEAKVACLEAKVAKLEAKVAC
e) KRGGKRGGK-(*palm*)
f) *palm*-VAKLEAKVACLEAKVACKG K G
g) *palm*-VAKLEAKVACLEAKVAKLEAKVACKG KG
h) *palm* -PrP
i) *palm* -PrP fragment

optionally, the peptide side chains at one or more positions being protected by protections groups.

33. A conjugate according to claim 32, wherein K is selectively side-chain deprotected compared to the other protected amino acid residues present in the peptide.

34. A conjugate according to any of claims 1-33 further comprising a marker.

35. A conjugate according to claim 34, wherein the marker is a fluorescent molecule, biotin, avidin, streptavidin, chemiluminescent molecule and the like.

36. A conjugate according to any of the preceding claims for use in the production of antibodies.

37. A conjugate according to claim 36 for use in the production of antibodies specific for PrP^{Sc}.

38. A conjugate according to any of the preceding claims for use in medicine.

39. A conjugate according to any of the preceding claims for use as a vaccine.

40. A conjugate according to any of the preceding claims for use in High-Throughput Screening.

41. A conjugate according to any of the preceding claims for use as direct probes for detection of PrP^{Sc}.

42. A conjugate according to any of the preceding claims for use as reporter substances in assays for the detection of PrP^{Sc}.

43. A method for the production of antibodies against PrP^{Sc}, the method comprising immunizing an animal with a conjugate according to claim 1-42.

44. A method according to claim 43, wherein the animal is selected from the group consisting of mice, rats, rabbits and poultry.

45. A method according to claim 43 or 44 for the production of monoclonal antibodies.

46. A method according to claim 43 or 44 for the production of polyclonal antibodies.

47. An antibody against PrP^{Sc} obtainable by the method claimed in any of claims 43-46.

48. An antibody according to claim 47, which when contacted with a mixture of PrP^{Sc} and PrP^{C} recognizes PrP^{Sc} without substantially recognizing PrP^{C}.

49. An antibody according to claim 47 or 48 for use in High-Throughput Screening.

50. A method for detection of PrP^{Sc} in a sample comprising

i) optionally, treating the sample with Proteinase K
ii) contacting the sample with an antibody according to claim 45 or 46,
iii) detecting any PrP^{Sc} which is bound to the antibody.

51. A method according to claim 50, wherein step i) is omitted.

52. A method according to claim 50 or 51, wherein the sample is of animal origin.

53. A method according to claim 52, wherein the sample is from a cow, a sheep, a monkey, a human, a pig, poultry, a mouse, a rat, a hamster, a Rocky Mountain Dear species or other mammals.

54. A method according to claim 53, wherein the sample is a body fluid sample selected

from the group consisting of blood, plasma, serum, urine, lymph, cerebrospinal fluid, saliva and tear fluid.

55. A method according to claim 53, wherein the sample is a body tissue sample selected from the group consisting of brain, muscles, lymphoid tissues, spinal cord, bone marrow, nerve tissue and blood cells.

56. A method according to claim 54 or 55, wherein the sample is from a living animal.

57. A method for identifying PrP^{Sc} by means of a substance which undergoes conformational change when contacted with PrP^{Sc}, the method comprising
i) incubation of the substance in a structure-relaxing solvent with PrP^{Sc},
ii) measuring any conformational change of the substance by conformation-specific antibodies or by detection of changes in the fluorescence of an environmentally sensitive fluorophore coupled to the substance.

58. A method according to claim 57, wherein the conformation-specific antibodies are defined in claim 47 or 48.

59. A pharmaceutical composition comprising a conjugate according to any of claims 1-42.

60. A pharmaceutical composition comprising an antibody according to any of claims 47 or 48.

61. A vaccine composition comprising a conjugate according to any of claims 1-42.

62. A vaccine composition comprising an antibody according to any of claims 47 or 48.

63. A method for treating and/or preventing Creutzfeldts-Jakobs disease, kuru, Gerstmann-Straussler-Sheinker disease, fatal familial insomnia and transmissible spongiform encephalopathies, such as bovine spongiform encephalopathy in cattle, scrapie in sheep, chronic wasting disease in deer and elk and transmissible encephalopathies in mink, cat and other animals, the method comprising administering to an animal an effective amount of a conjugate according to any of claims 1-42.

64. A method for treating and/or preventing Creutzfeldts-Jakobs disease, kuru, Gerstmann-Straussler-Sheinker disease, fatal familial insomnia and transmissible spongiform encephalopathies, such as bovine spongiform encephalopathy in cattle,

scrapie in sheep, chronic wasting disease in deer and elk and transmissible encephalopathies in mink, cat and other animals, the method comprising administering to an animal an effective amount of an antibody according to any of claims 47 or 48.

SEQUENCE LISTING

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<170> PatentIn version 3.1

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85 90 95

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100 105 110

Pro Ser Lys Pro Lys Thr Asn Met Lys His Val Ala Gly Ala Ala Ala
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Phe Thr Glu Thr Asp Ile Lys Met Met Glu Arg Val Val Glu Gln Met
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Cys Ile Thr Gln Tyr Gln Arg Glu Ser Gln Ala Tyr Tyr Gln Arg Gly
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35 40 45

Gly Asn Arg Tyr Pro Pro Gln Gly Gly Gly Gly Trp Gly Gln Pro His
50 55 60

Gly Gly Gly Trp Gly Gln Pro His Gly Gly Gly Trp Gly Gln Pro His
65 70 75 80

Gly Gly Gly Trp Gly Gln Pro His Gly Gly Gly Gly Trp Gly Gln Gly

85 90 95

Gly Ser His Ser Gln Trp Asn Lys Pro Ser Lys Pro Lys Thr Asn Met
100 105 110

Lys His Val Ala Gly Ala Ala Ala Ala Gly Ala Val Val Gly Gly Leu
115 120 125

Gly Gly Tyr Met Leu Gly Ser Ala Met Ser Arg Pro Leu Ile His Phe
130 135 140

Gly Asn Asp Tyr Glu Asp Arg Tyr Tyr Arg Glu Asn Met Tyr Arg Tyr
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Pro Asn Gln Val Tyr Tyr Arg Pro Val Asp Arg Tyr Ser Asn Gln Asn
165 170 175

Asn Phe Val His Asp Cys Val Asn Ile Thr Val Lys Gln His Thr Val
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Thr Thr Thr Thr Lys Gly Glu Asn Phe Thr Glu Thr Asp Ile Lys Ile
195 200 205

Met Glu Arg Val Val Glu Gln Met Cys Ile Thr Gln Tyr Gln Arg Glu
210 215 220

Ser Gln Ala Tyr Tyr Gln Arg Gly Ala Ser Val Ile Leu Phe Ser Ser
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Pro Pro Val Ile Leu Leu Ile Ser Phe Leu Ile Phe Leu Ile Val Gly
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<213> Homo sapiens

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20 25 30

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35 40 45

Tyr Pro Pro Gln Gly Gly Gly Gly Trp Gly Gln Pro His Gly Gly Gly
50 55 60

Trp Gly Gln Pro His Gly Gly Gly Trp Gly Gln Pro His Gly Gly Gly
65 70 75 80

Trp Gly Gln Pro His Gly Gly Gly Trp Gly Gln Gly Gly Gly Thr His
85 90 95

Ser Gln Trp Asn Lys Pro Ser Lys Pro Lys Thr Asn Met Lys His Met
100 105 110

Ala Gly Ala Ala Ala Ala Gly Ala Val Val Gly Gly Leu Gly Gly Tyr
115 120 125

Met Leu Gly Ser Ala Met Ser Arg Pro Ile Ile His Phe Gly Ser Asp
130 135 140

Tyr Glu Asp Arg Tyr Tyr Arg Glu Asn Met His Arg Tyr Pro Asn Gln
145 150 155 160

Val Tyr Tyr Arg Pro Met Asp Glu Tyr Ser Asn Gln Asn Asn Phe Val
165 170 175

His Asp Cys Val Asn Ile Thr Ile Lys Gln His Thr Val Thr Thr Thr
180 185 190

Thr Lys Gly Glu Asn Phe Thr Glu Thr Asp Val Lys Met Met Glu Arg
195 200 205

Val Val Glu Gln Met Cys Ile Thr Gln Tyr Glu Arg Glu Ser Gln Ala
210 215 220

Tyr Tyr Gln Arg Gly Ser Ser Met Val Leu Phe Ser Ser Pro Pro Val
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<400> 12

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Leu Phe Ser

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Met Lys His Val
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<400> 15

Gly Ser Asp Tyr Glu Asp Arg Tyr Tyr Arg Glu Asn Met His Arg Tyr
1 5 10 15

Pro Asn Gln

<210> SEQ ID No.16

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<400> 16

Tyr Met Leu Gly Ser Ala Met Ser Arg Pro Leu Ile His Phe Gly Ser
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Gln Val Tyr Tyr Arg Pro
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<400> 17

Ser Ala Met Ser Arg Pro Leu Ile His Phe Gly Ser Asp Tyr Glu Asp
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Arg Tyr Tyr Arg
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<213> Bos taurus

<400> 18

Gly Gly Thr His Gly Gln Trp Asn Lys Pro Ser Lys Pro Lys Thr Asn
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Met Lys His Val
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<210> SEQ ID No.19

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<212> PRT

<213> Bos taurus

<400> 19

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<212> PRT

<213> Bos taurus

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Gln Val Tyr Tyr Arg Pro
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<213> Bos taurus

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Thr Asn Met Lys His Val Ala Gly Ala Ala Ala Ala Gly Ala Val Val
20 25 30

Gly Gly Leu Gly Gly Tyr Met Leu Gly Ser Ala Met Ser Arg Pro Leu
35 40 45

Ile His Phe Asp Cys Val Asn Ile Thr Val Lys Glu His Thr Val Thr
50 55 60

Thr Thr Thr Lys Gly Glu Asn Phe Thr Glu Thr Asp Ile Lys Met Met

65 70 75 80

Glu Arg Val Val Glu Gln Met Cys Ile Thr Gln Tyr Gln Arg Glu Ser
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<211> 55

<212> PRT

<213> Bos taurus

<400> 27

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His Thr Val Thr Thr Thr Thr Lys Gly Glu Asn Phe Thr Glu Thr Asp
20 25 30

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Gln Arg Glu Ser Gln Ala Tyr
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<213> Bos taurus

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Gln Arg Glu
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<213> Bos taurus

<400> 29

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Gln

<210> SEQ ID No.25E

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<213> Bos taurus

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Ile Lys Met Met Glu Arg Val Val Glu Gln Met Cys Ile Thr Gln
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<210> SEQ ID No.25F

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<213> Bos taurus

<400> 31

Ser Arg Pro Leu Ile His Phe Asp Cys Val Asn Ile Thr Val Lys Glu
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His Thr Val Thr Thr Thr Thr Lys Gly Glu Asn Phe Thr Glu Thr Asp
20 25 30

Ile Lys Met Met Glu Arg Val Val Glu Gln Met Cys Ile Thr
35 40 45

<210> SEQ ID No.26

<211> 56

<212> PRT

<213> Bos taurus

<400> 32

Gly Gly Trp Gly Gln Pro His Gly Gly Gly Gly Trp Gly Gln Gly Gly
1 5 10 15

Thr His Gly Gln Trp Asn Lys Pro Ser Lys Pro Lys Thr Asn Met Lys
20 25 30

His Val Ala Gly Ala Ala Ala Ala Gly Ala Val Val Gly Gly Leu Gly
35 40 45

Gly Tyr Met Leu Gly Ser Ala Met
50 55

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<211> 12

<212> PRT

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<400> 33

Gly Gln Gly Gly Thr His Gly Gln Trp Asn Lys Pro
1 5 10

<210> SEQ ID No.28

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Gly Gln Trp Asn Lys Pro Ser Lys Pro Lys Thr Asn
1 5 10

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<211> 18

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Gly Gly Leu Gly Gly Tyr Met Leu Gly Ser Ala Met Ser Arg Pro Leu
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Ile His

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<212> PRT

<213> Bos taurus

<400> 36

Leu Gly Gly Tyr Met Leu Gly Ser Ala Met Ser Arg Pro Leu Ile His
1 5 10 15

Phe Gly Ser

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<400> 37

Arg Glu Ser Gln Ala Tyr Tyr Gln Arg Gly Ala Ser
1 5 10

<210> SEQ ID No.32

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<212> PRT

<213> Bos taurus

<400> 38

Gly Gly Gly Gly Trp Gly Gln Gly Gly Thr His Gly Gln Trp Asn Lys
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<210> SEQ ID No.33

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<213> Bos taurus

<400> 39

Gly Ser Asp Tyr Glu Asp Arg Tyr Tyr Arg Glu Asn Met His Arg Tyr
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Pro Asn Gln

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Cys Gly Ser Asp Tyr Glu Asp Arg Tyr
1 5

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Tyr Tyr Arg Pro Val Asp Gln Tyr Ser
1 5

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<212> PRT

<213> Bos taurus

<400> 42

Thr Gln Tyr Gln Arg Glu Ser Gln Ala Tyr Tyr
1 5 10

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<211> 17

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<213> Bos taurus

<400> 43

Gln Arg Gly Ala Ser Val Ile Leu Phe Ser Ser Pro Pro Val Ile Leu
1 5 10 15

Leu

<210> SEQ ID No.38

<211> 14

<212> PRT

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Tyr Tyr Arg Pro Val Asp Gln Tyr Ser Asn Gln Asn Asn Phe
1 5 10

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<211> 12

<212> PRT

<213> Bos taurus

<400> 45

Ile Thr Gln Tyr Gln Arg Glu Ser Gln Ala Tyr Tyr
1 5 10

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<211> 12

<212> PRT

<213> Bos taurus

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Thr Lys Gly Glu Asn Phe Thr Glu Thr Asp Ile Lys
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<212> PRT

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Gly Ser Asp Tyr Glu Asp Arg Tyr
1 5

<210> SEQ ID No.42

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Asp Tyr Glu Asp Arg Tyr Tyr Arg Glu
1 5

<210> SEQ ID No.43

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<212> PRT

<213> Bos taurus

<400> 49

Tyr Tyr Arg Pro Val Asp Gln Tyr Ser
1 5

<210> SEQ ID No.44

<211> 14

<212> PRT

<213> Bos taurus

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Thr Gln Tyr Gln Arg Glu Ser Gln Ala Tyr Tyr Gln Arg Gly
1 5 10

Fig. 1

Fig. 2

Fig. 3

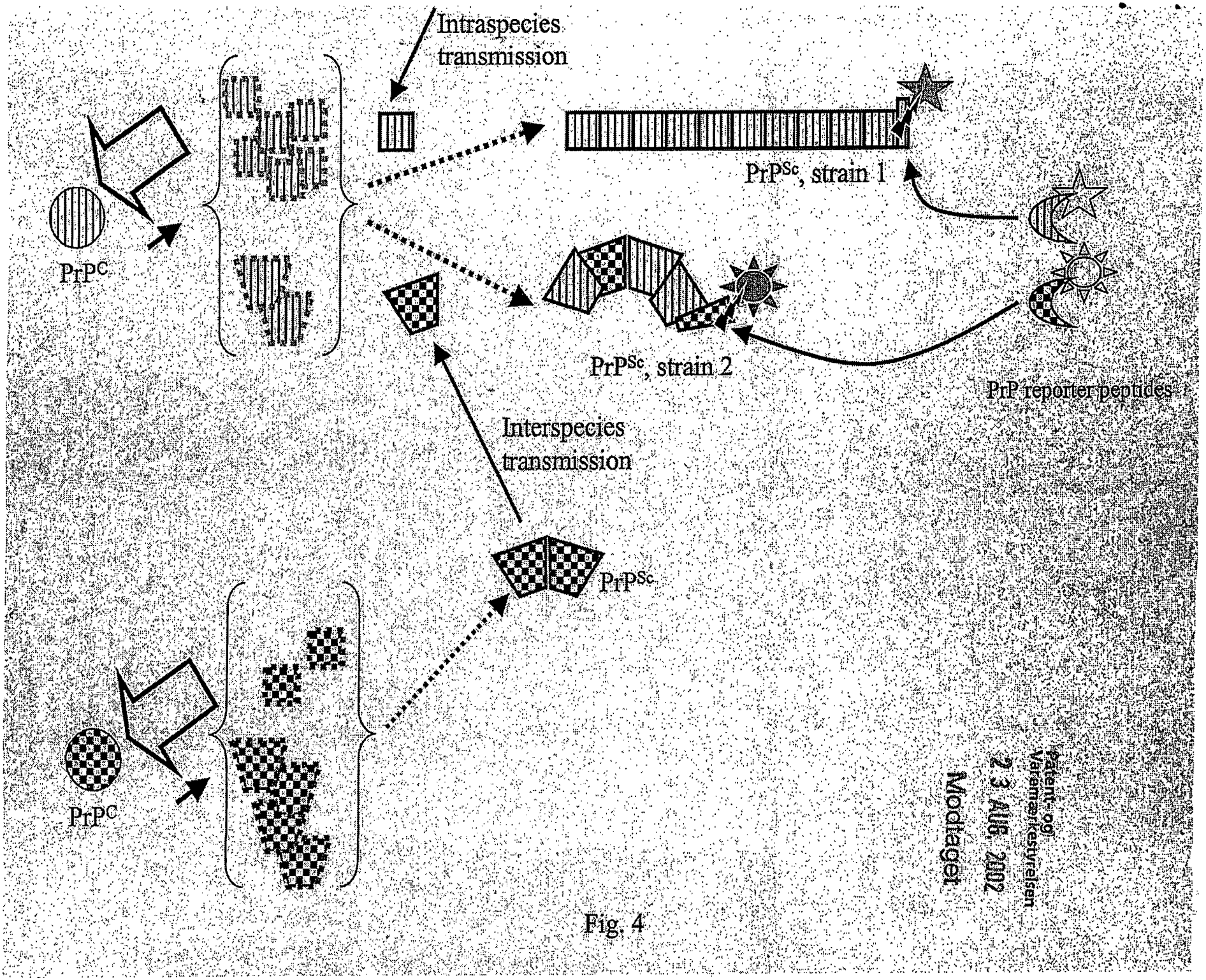

Fig. 4